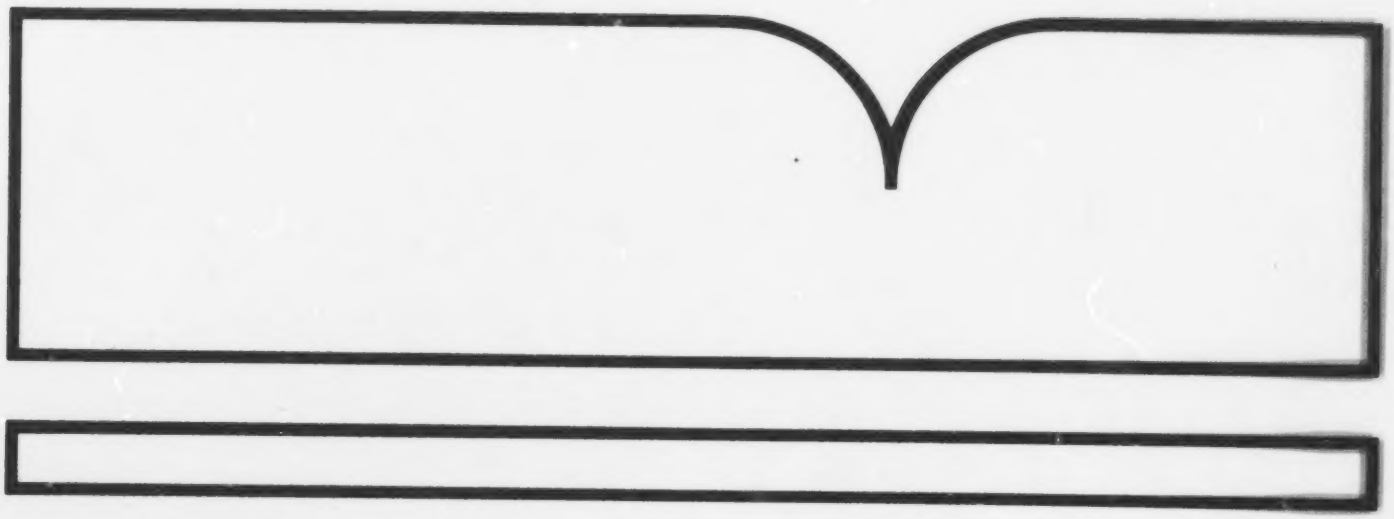
PB86-176054

Waterborne Viral Gastroenteritis

(U.S.) Health Effects Research Lab.
Cincinnati, OH

1986



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1. REPORT NO. EPA/600/J-86/007	2.	3. PB86-176054
4. TITLE AND SUBTITLE Waterborne Viral Gastroenteritis (Journal Version)		5. REPORT DATE 1986
		6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) Fred P. Williams, Jr. and Elmer W. Akin		8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS Toxicology and Microbiology Division Health Effect Research Laboratory, ORD U.S. Environmental Protection Agency Cincinnati, OH 45268		10. PROGRAM ELEMENT NO. ABMC1A
		11. CONTRACT/GRANT NO. Inhouse
12. SPONSORING AGENCY NAME AND ADDRESS Health Effects Research Laboratory Office of Research and Development U.S. Environmental Protection Agency Research Triangle Park, North Carolina 27711		13. TYPE OF REPORT AND PERIOD COVERED Journal
		14. SPONSORING AGENCY CODE EPA-600/11

15. SUPPLEMENTARY NOTES
P.O. Elmer Akin Journal of American Water Works Association
Vol. 78, No. 1, 34-39, January 1986

16. ABSTRACT

In the study of human gastroenteritis, the use of electron microscopy and related techniques has led to the identification of new viral agents which had previously escaped detection by routine cell-culture procedures. Efforts to characterize and further study these agents are currently being made by researchers in many areas of the world. Two of the best known agents, rotavirus and Norwalk virus, have now been implicated in waterborne outbreaks of this illness. Another virus, the Snow Mountain agent, was first identified from the investigation of one waterborne gastroenteritis outbreak.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

18. DISTRIBUTION STATEMENT Release to public	19. SECURITY CLASS (This Report) unclassified	21. NO. OF PAGES 8
	20. SECURITY CLASS (This page) unclassified	22. PRICE $9.95

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

EPA/600/J-86/007
JOURNAL ARTICLE

Waterborne Viral Gastroenteritis

Fred P. Williams Jr. and Elmer W. Akin

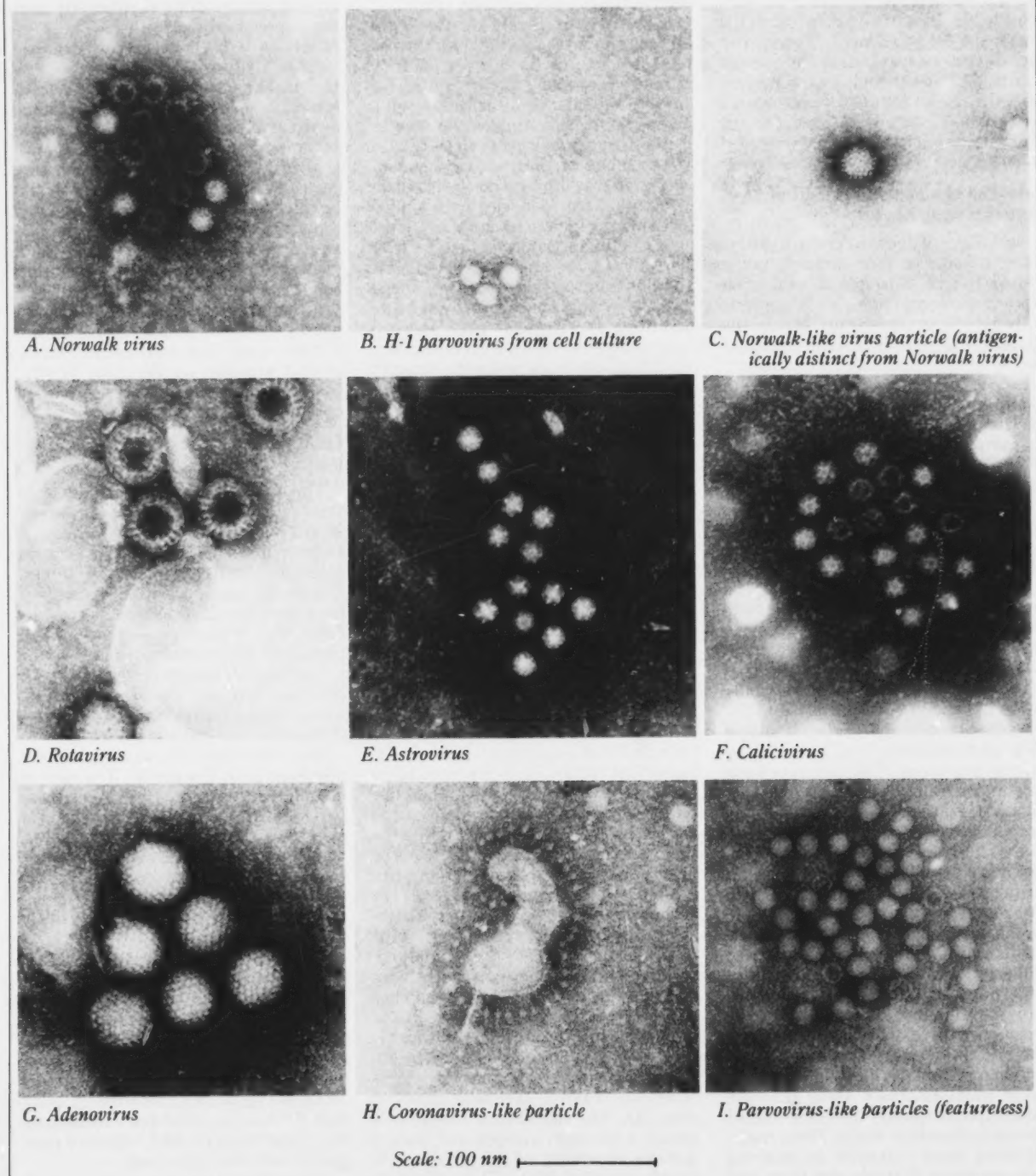
In studying the causes of human gastroenteritis, electron microscopy and related techniques have led to the identification of new viral agents that had previously escaped detection by routine cell culture procedures. Efforts to characterize and study these agents further are currently being made by researchers in many areas of the world. Two of the best-known agents, rotavirus and Norwalk virus, have been implicated in waterborne outbreaks of this illness. Another virus, the Snow Mountain agent, was first identified from the investigation of one waterborne gastroenteritis outbreak.

Acute gastroenteritis is second only to the common cold as the most frequent illness affecting the population of the United States.[1] In a recent six-year prospective epidemiological study, researchers found that the study population, representing ages from less than 1 year to more than 50 years, would experience an average of 1.2 episodes of enteric illness each year.[2] Although rarely fatal in the United States, gastroenteritis imposes a degree of misery as well as loss of time from work, school, and other planned activities. In the developing countries of the world, diarrhea is a major cause of death, especially in very young children.

There are many causes of gastroenteritis, including microorganisms in the bacterial, parasitic, and viral categories. Enteric bacterial pathogens have long been identified, and the major role of drinking water in their dissemination was known by the turn of the century. More recently, cysts of the parasite *Giardia* have been recognized as a significant cause of waterborne gastroenteritis in the United States.[3]

Enteric viral pathogens, although suspected for decades, were not isolated and identified until the development of in vitro cell culture procedures about 1950. In the early 1950s, Robbins and coworkers isolated poliovirus from the feces of infected humans in cell cultures.[4] This discovery proliferated the investigation of fecal and other clinical specimens for viral agents of infectious disease using the newly available cell culture techniques. Within a few years, these studies had isolated and identified most of the enteric viruses known today. About 100 distinct virus serotypes were isolated in large numbers from stools of individuals with gastroenteritis and other diseases as well as from people without any disease symptoms, i.e., asymptomatic infections.

Hepatitis had been recognized as a viral disease that could be waterborne as determined through epidemiological investigation. However, isolation of the responsible viruses did not yield to the standard cell culture procedures. For many years hepatitis viruses had the distinction of being the only enteric viruses widely accepted as producing waterborne disease, yet the viruses remained undetectable in the laboratory. Only now are practical procedures becoming available to isolate and cultivate hepatitis A virus (HAV) in vitro.[5]



A. Norwalk virus

B. H-1 parvovirus from cell culture

C. Norwalk-like virus particle (antigenically distinct from Norwalk virus)

D. Rotavirus

E. Astrovirus

F. Calicivirus

G. Adenovirus

H. Coronavirus-like particle

I. Parvovirus-like particles (featureless)

Scale: 100 nm

Figure 1. Electron micrographs of different types of virus particles *(the distinctive morphological features of the viruses can be seen by negatively staining the virus preparations with a dark stain; occasionally, the surrounding stain will penetrate a virus particle and reveal the core of the virus—such penetrated particles can be seen in A, F, and I and are particularly evident in D; the micrographs were taken using a 2 percent phosphotungstic acid, pH 7, as the negative stain with magnification of 100 000 X)*

The occurrence of waterborne gastroenteritis outbreaks had also been confirmed by epidemiological investigation. However, no significant association with the known enteric viruses had been achieved by 1965, the year in which the first major international symposium on the waterborne transmission of viruses was held.[6] Even though gastroenteritis was the most frequently recognized waterborne disease worldwide, the role of viruses as etiological agents remained an enigma.

Detection of two major groups of gastroenteritis viruses

Outbreaks of acute infectious gastroenteritis had repeatedly occurred in the United States although identification procedures for commonly known enteric pathogens had failed to isolate the etiological agent from diarrheal stools of the victims. In the early 1970s, investigators at the National Institutes of Health applied a new procedure, immune electron microscopy (IEM), to the study of a gastroenteritis outbreak in Norwalk, Ohio. This investigation detected aggregates of small (27–32-nm) virus particles when stool filtrates from people with the illness were preincubated with convalescent sera from infected volunteers.[7] The virus identified by this investigation, the Norwalk virus, is known to be responsible for outbreaks of gastroenteritis in humans of all ages. It became representative of a new group of small enteric viruses that could be detected with the electron microscope but could not be isolated in cell cultures.

A second major virus type associated with gastroenteritis was detected by electron microscopy (EM) about the same time in Australia.[8] Within a brief time, laboratories around the world identified morphologically identical virus particles in the diarrheal stools of infants and young children. This virus, rotavirus, is considerably larger (approximately 70 nm) than Norwalk virus, is present in much larger concentrations in the stools of infected individuals, and is associated primarily with winter gastroenteritis in infants and young children.

In the 10 years subsequent to the detection of these two types of viruses by EM and IEM, other techniques, i.e., radioimmunoassay (RIA) and enzyme-linked immunosorbent assay (ELISA), have helped to identify these and related viruses in diarrheal stools. These methods were more adaptable to assaying large numbers of stools and were also effective in detecting serum conversions in infected individuals. Although epidemiological studies were made possible by these procedures, comprehensive characterization of the two virus groups has awaited the development of successful cell culture procedures. Suitable cell culture procedures have now been developed for the cultivation of human rotaviruses and have allowed substantial progress in their characterization.[9-13] Unfortunately, similar success has not been achieved with Norwalk virus.

Norwalk virus (Figure 1A) was originally categorized as being "parvovirus-like" because of its buoyant density, relative size, and stability.[14,15] It has since become apparent that this agent is not a parvovirus. Unlike the smaller featureless parvovirus particles (Figure 1B), particles of Norwalk virus have a characteristic, although not well-defined, structure. Virus particles with similar morphological characteristics, but which appear to be antigenically distinct from Norwalk, are also known to exist (Figure 1C). The Hawaii agent represents such a virus.[16,17] Viruses of this type are better described as "Norwalk-like" than as "parvovirus-like" and have been included with Norwalk virus under the category "small round structured virus" in one interim attempt at classification.[17] It is not known how many Norwalk-like viruses exist.

The nucleic acid content of the Norwalk virus is not known. A preliminary analysis of the protein structure revealed the presence of a single protein with a mol wt of 59 000.[18] The result is similar to that found with calicivirus, a virus containing ribonucleic acid (RNA) that is similar morphologically to Norwalk. Little other structural information is known about the virus, and it is clear that much additional research is needed before the Norwalk-like viruses can be adequately characterized and classified.

Rotavirus, on the other hand, has now been classified as a separate genus in the Reoviridae family. This family includes the reoviruses and the orbiviruses. The rotavirus name was derived from the distinctive wheel-like appearance (the Latin *rota* means wheel) of many of the virus particles (as shown in Figure 1D). There are at least four distinct serotypes of human rotavirus that are distinguishable by plaque reduction or tube neutralization tests.[12,19] Other immunological methods, such as ELISA, can differentiate other rotavirus groupings that have been termed subgroups.[20] The subgroup antigens are distinct from the serotype antigens and are coded by different viral genes. At least two rotavirus subgroups (I and II) have been identified thus far. The rotaviruses share, as a group, a common antigen and have a genome consisting of 11 segments of double-stranded RNA. Electrophoresis of the rotavirus genome has resulted in some variation, but two basic electrophoretic patterns have been identified—a "short" pattern and a "long" pattern.[21]

Recently, virus particles have been reported in several species, including man, that are morphologically indistinguishable from other rotavirus particles, but the particles lack the common group antigen.[22-27] Although they do possess a segmented, double-stranded RNA genome, their electrophoretic migration patterns differ significantly from those previously identified. These viruses have been termed "pararotaviruses," "atypical rotaviruses," or "rotavirus-like agents" by different investigators. Based on antigenic and genomic analyses, there appear to be at least two separate groups of these common-antigen-lacking viruses.[28] It has been suggested that these atypical viruses should be categorized as group B and group C rotaviruses with the original common-antigen viruses being the group A rotaviruses. There is recent evidence that additional groups exist.[29-31]

Although success has been achieved in the cell culture propagation of certain human rotaviruses, practical procedures are not available for the assay of viral gastroenteritis agents as a group in environmental samples.

Other viruses associated with gastroenteritis

The application of EM techniques to the search for viral agents of gastroenteritis has uncovered a variety of additional particle types that escaped detection by cell cultivation approaches. The morphologically distinct agents that have been identified include astrovirus, calicivirus, and enteric adenovirus (Figures 1E–G). Pleomorphic particles bearing a resemblance to coronavirus have also been detected, but the etiologic significance of these coronavirus-like particles (Figure 1H) is unclear at present.

Featureless small round particles have also been seen (Figure 1I). The lack of distinctive characteristics makes these particles difficult to identify by EM alone. The Wollan, Ditchling, cockle, and Parramata agents, detected in separate outbreaks of gastroenteritis, fit into this category.[17] Because of their small size, these four specific agents are likely to be parvoviruses.

The use of IEM, a technique that detects antibody-aggregated or coated virus particles, unfortunately tends to obscure the characteristic structural detail of viruses. Agents detected by this method can be difficult to classify morphologically. Marin agent and the Snow Mountain agent, both detected by using IEM,[32,33] have revealed few distinguishing characteristics with which to categorize them other than size.

Other distinctive virus particles, such as minireovirus or minirotavirus, and several agents detected in Japan (including Otafuki and Sapporo agents) have been described. It would appear from published micrographs that these agents fit with Norwalk and Hawaii viruses under the category of "small round structured virus."[17] It is possible, how-

TABLE 1

Four waterborne outbreaks associated with Norwalk virus

Location (Date)	Number of Ill Persons	Remarks	Norwalk Virus Association
Pierce County, Wash. (1978).[36]	430*	Elementary school water system contaminated—likely back-siphonage through a cross connection between well and septic tank	Four-fold or greater rise in antibody titer in 2 of 3 individuals
Monroe County, Pa. (1978)[37]	193	Summer camp water system contaminated—inadequate chlorination	Four-fold or greater rise in antibody titer in 3 of 3 individuals
Rome, Ga. (1980)[38]	1500	Municipal water system likely contaminated by a textile plant water system	Four-fold or greater rise in antibody titer in 12 of 19 individuals
Tate, Ga. (1982)[39]	500	Older community water system contaminated—system had several deficiencies and possible sources of contamination	Four-fold or greater rise in antibody titer in 20 of 22 individuals

*Calculated from reported attack rate

ever, that future studies will find that at least some of these agents can be morphologically distinguished from the others.

It has not been conclusively determined that all these agents are responsible for gastrointestinal illness in humans. Although these particles are usually found in the stools of afflicted individuals, they also have been observed in the stools of asymptomatic individuals. Clearly, further research is required before their association with disease can be fully understood.

Outbreaks of waterborne viral gastroenteritis

Conclusive evidence of the waterborne nature and the viral etiology of gastroenteritis outbreaks is difficult to obtain. Epidemiological investigations are often limited in scope and frequently occur after the fact. In about half the reported outbreaks of waterborne disease, the etiological agent is never identified.[34] Adequate laboratory support and appropriate specimens and water samples are often unavailable or are limited in number. In many outbreaks, the available data indicate, but not necessarily overwhelmingly, that a specific virus was the etiological agent and that water was the route of transmission. There is, however, a growing body of epidemiological data that implicate at least three viral agents in outbreaks of waterborne gastroenteritis. Norwalk virus has been associated with most of these outbreaks; however, there have been a number of outbreaks of waterborne disease in which rotavirus has been implicated. Another virus, the Snow Mountain agent, was first identified from the investigation of one waterborne disease in Colorado.

Norwalk virus outbreaks. In a recent review of outbreaks of acute nonbacterial gastroenteritis investigated by the Centers for Disease Control during the period 1976–80, 31 of 74 (42 percent) of the outbreaks were attributed to Norwalk virus.[35] Of these 31, and seven additional outbreaks examined at the National Institute of Health, water was implicated as the vehicle of transmission in 13 outbreaks. Municipal water systems were implicated in two outbreaks, semipublic water supplies in seven outbreaks, stored water on cruise ships in two outbreaks, and recreational swimming was involved in two outbreaks.

Table 1 lists four Norwalk virus-associated outbreaks described in the literature that involved drinking water supplies. In each outbreak, epidemiological analysis, consisting of questionnaires or telephone surveys, associated the illness with drinking water from a particular water system. The determination of a Norwalk virus etiology for these outbreaks was based on RIA examination of paired serum specimens and the exclusion of other pathogens.

The first outbreak listed occurred at an elementary school in the state of Washington in 1978. The illness afflicted 72 percent of approximately 600 students, teachers, and support personnel. A blocked septic tank entry port and an unfortunate modification of the school water system by maintenance personnel were responsible for the contamination of the water supply.

An outbreak in Pennsylvania, also in 1978, involved two summer sessions at a children's recreational camp. One hundred ninety-three cases of illness were reported. Subsequent investigation revealed fecal contamination and inadequate chlorination of the well water system.

An outbreak involving approximately 1500 ill individuals took place in Rome, Ga., in 1980. This outbreak involved a community water system, and cases were concentrated near a textile plant. The plant had its own nonpotable water system that was found to be contaminated. The nonpotable system had two connections with the community water system. Inadequate single gate valves separating the two systems apparently allowed contamination of the community system.

In 1982, another outbreak in Georgia afflicted about 500 people living in a small rural community. The people were served by a water system built in 1926 that, upon investigation, had several possible contaminated water sources. One source, a spring, was located within a hog pen and was susceptible to groundwater runoff from nearby homes with septic tanks.

In addition to the four epidemiological reports summarized in Table 1, a Norwalk-associated waterborne outbreak has provided the clinical specimens for a recent serological study involving teenagers.[40] The outbreak, which occurred at a recreational camp in western North Carolina in 1979, allowed examination of serological responses among a younger age group than has been feasible through volunteer studies.

Rotavirus outbreaks. Although rotavirus is primarily considered to be a pathogen for infants and young children, it has been associated with acute gastrointestinal illness in adults, and several outbreaks among the elderly, in particular, have been attributed to this agent.[41,42] Water-related outbreaks associated with rotavirus have also been described in the literature. These outbreaks have occurred in widely separated areas of the world. A list of these outbreaks, which occurred in seven different countries including the United States, is presented in Table 2.

The first reported outbreak that appeared to be associated with rotavirus and water occurred in a small town in Sweden in 1977. Approximately 30 percent of the population was afflicted. Rotavirus particles were observed by electron microscopy in the stools of 17 of 36 acutely ill individuals. Since three rotavirus-containing stools were also found to contain 30 nm particles, it is possible that another virus had some involvement in the disease outbreak. Observations indicated that the town's water supply had been contaminated with sewage effluent.

A private Brazilian school's water supply was implicated in a 1980 outbreak

TABLE 2
Waterborne outbreaks associated with rotavirus

Location (Date)	Number of Ill Persons	Remarks	Rotavirus Association
Sweden (1977)[43]	3 172	Small town's water supply was evidently contaminated with sewage effluent	Rotavirus observed by EM in 17 of 36 stools
Rio de Janeiro, Brazil (1980)[44]	812	Private school's water contaminated—accidental pumping of water from a poorly protected shallow well was the apparent cause	Rotavirus detected by EM and ELISA in 11 of 19 stools
Eagle-Vail-Avon, Colo. (1981)[45]	1 500*	Two small communities' water supply involved—source contamination, chlorinator failure, and severely channeled filter beds appeared responsible	Stool rotavirus observed by EM or serum conversions by IEM and RIA in 5 of 7 individuals
Soviet Union (1981)*[46]	173	Contamination of the municipal water system responsible for outbreak at a settlement	Rotavirus detected in 10 of 24 stools
East Germany (1981–82)[47]	11 600	Large town's wells located near river were flooded—river water was known to be contaminated	Rotavirus observed by EM in 7 of 14 stools
Lanzhou and Jinzhou, China (1982–83)[31]	13 311	Two separate outbreaks involving atypical rotavirus—investigation of the Jinzhou outbreak indicated a contaminated water supply	Rotavirus observed by EM in 101 of 106 stools
Israel (1984)*[48]	Unknown	A summary of waterborne outbreaks in Israel (1976–80) reported one outbreak involving a community water supply and rotavirus	Rotavirus identified in 3 of 17 stools

*Date of report

that involved both rotavirus and *Shigella sonnei*. The school had accidently been pumping water from a shallow well that was known to contain high coliform counts. Rotavirus was detected by both IEM and ELISA in 11 of 19 illness stools (*S. sonnei* was found in 4 of these 11 and in 6 others).

In 1981, an outbreak of gastroenteritis occurred in two small Colorado communities served by the same water district. The outbreak in Eagle-Vail and Avon was jointly investigated by the Colorado Department of Health and researchers at both the US Environmental Protection Agency and the University of Massachusetts. Telephone surveys indicated an attack rate of 32 percent, and perhaps 1500 individuals were ill during the outbreak. The illness was statistically associated with water consumption. Examination of stools by EM and paired sera by IEM and RIA revealed rotavirus infection in 5 of 7 ill individuals. Tests for *Salmonella, Shigella*, enterotoxigenic *E. coli, Campylobacter*, and Norwalk virus were all negative. A chlorinator failure at the water district's treatment plant occurred just prior to the outbreak. The primary water source at the time was a river. It was found that another community's sewage treatment plant had been discharging sludge into a creek that emptied into this river upstream from the Eagle-Vail-Avon intake. In addition to the chlorinator failure, the Eagle-Vail-Avon water treatment plant was found to have severely channeled filter beds. There was no chemical or physical pretreatment of the water prior to filtration.

A 1981 report from the Soviet Union described an outbreak at a settlement involving at least 173 cases of gastroenteritis. An epidemiologic investigation indicated contamination of the municipal water system. Rotavirus was found in the feces of 10 of 24 patients.

During late 1981 and early 1982, an outbreak of gastroenteritis occurred at a large East German town situated along a river. At least 11 600 cases of the illness were reported between November and January. The outbreak began in areas receiving water from wells located near the river bank and coincided with the flooding of these wells. In addition, the chlorination practice was being changed at that time. Rotavirus was detected in 7 of 14 stools examined by EM. The virus was also found in sewage samples.

In late 1982 and early 1983, two epidemics of acute diarrhea occurred in two coal mining areas in China near Lanzhou and Jinzhou. The outbreaks involved more than 12 000 adults and were the first to be associated with rotavirus without the common group antigen. Information reported on the Jinzhou outbreak indicated that the water supply had been fecally contaminated. The pattern of spread during the outbreak indicated that the illness was initially transmitted through the water supply, and individuals using deep well water were much less likely to have been afflicted. Rotavirus particles were detected in 101 of 106 cases of illness. The virus appeared to be distinct from other atypical rotaviruses, and it was proposed that the virus implicated in these outbreaks belongs to a fourth group of rotaviruses.

Finally, in a 1984 summary of waterborne outbreaks occurring in Israel during 1976–80, rotavirus was identified in one outbreak involving a community water supply. The virus was detected in the stools of 3 of 17 patients. *Escherichia coli* and *Streptococcus faecalis* were detected in the water.

The Snow Mountain agent outbreak. Investigation of gastroenteritis cases at a camp in Colorado in 1976 provided convincing evidence of a viral waterborne outbreak.[49] Spring water contaminated by septic tank leakage was distributed throughout the camp, resulting in more than 400 cases of disease. Chlorination was the only treatment provided for the drinking water, and this treatment had been interrupted prior to the outbreak. A 27-nm virus, later referred to as Snow Mountain agent, was identified by IEM in 2 of 5 stools from ill persons. Three individuals were shown by IEM to have serum conversions to the virus particles.[33] Although rotavirus was detected in another stool, the shedding individual had a preexisting antibody, and a serum conversion to rotavirus could not be detected. Eight other serum pairs from the outbreak were negative when examined with this shedding individual's stool using counter-immunoelectro-osmophoresis.

The Snow Mountain agent is antigenically distinct from both Norwalk and Hawaii viruses but appears to be morphologically similar when observed by IEM.

Summary

A number of newly identified viral gastroenteritis agents have been reported since the early 1970s. Of these, Norwalk virus, rotavirus, and the Snow Mountain agent have been implicated in waterborne outbreaks of the illness. Since these agents could not be readily detected using routine cell culture procedures, progress in their characterization has been slow. Unfortunately, understanding of their environmental significance remains fragmentary, and their detection in water supplies depends to a great extent on the development of more prac-

tical and efficient methods for their identification in water supplies.

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